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Preparation and Properties of Glucoconringiin, the Precursor of the Thyreostatic 5,5-Dimethyl-2-Oxazolidinethione

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The mustard oil glucoside glucoconringiin is present according to Kjær *et al.*[1] in several *Cochlearia* spec. which are rather common in the Finnish flora. In the course of investigations with goitrogens in plants it seemed therefore of interest to isolate this glucosidic precursor of the thyreostatic and probably also goitrogenic 5,5-dimethyl-2-oxazolidinethione.

Some years ago Kjær *et al.*[1] and Schultz and Wagner [2] prepared and characterized glucoconringiin as its tetraacetyl derivative from seeds of *Conringia orientalis* (L.) Andrz. and showed that the deacetylated glucoside which they obtained in amorphous form is split by myrosinase into sulphate, glucose and 5,5-dimethyl-2-oxazolidinethione. This heterocyclic compound, isolated and identified by Hopkins[3] twenty years ago from *C. orientalis* seeds, is formed by spontaneous cyclization from an intermediate 2-hydroxy-2-methylpropyl *iso*thiocyanate. Its thyreostatic effect was established by Astwood *et al.*[4] and was found comparable to that of (−)-5-vinyl-2-oxazolidinethione (goitrin).

The purpose of this paper is to report briefly the method for preparation and some properties of crystalline glucoconringiin, which unlike its tetraacetyl derivative has the advantage of being split readily by myrosinase and is therefore more suitable for physiological investigations.

Glucoconringiin was prepared by ion exchange on Dowex 2-X 4 of an extract from seeds of *Conringia orientalis* (L.) Andrz. Upon elution with K_2SO_4 solution it was obtained as a colourless syrup which crystallized upon long standing in the refrigerator. Two recrystallizations from 90 % ethanol yielded pure glucoconringiin as anhydrous potassium salt in white, short needles. Elementary analysis agreed with the composition $C_{11}H_{20}NO_{10}S_2K$. F 168°C (decomp., uncorr.), $[\alpha]_D^{21}$ −10.87° in H_2O). In agreement with earlier results [1,2] the formation of glucose, sulfate and 5,5-dimethyl-2-oxazolidinethione could be observed during enzymatic cleavage. The enzymatic process could be followed by UV spectroscopy: with increasing cleavage the maximum absorbance shifts from 230.5 mμ to 240 mμ (ε = 15 400), the maximum of 5,5-dimethyl-2-oxazolidinethione.

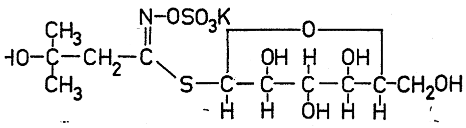
Spectrophotometric assay of the enzymatic cleavage of glucoconringiin: 0.0155 g of glucoconringiin was dissolved in 2 ml of a mixture of equal parts of myrosinase solution and phosphate buffer pH 6.8. In certain intervals 0.1 ml portions were taken off, diluted to 50 ml with water and the UV absorption measured between 220 mμ and 260 mμ against an equal blank dilution of myrosinase solution and phosphate buffer solution.

t min	UV-maximum mμ	Optical density	Mol.* extinction, ε
1	230.5	0.245	6 760
20	233	0.258	7 140
90	235	0.310	8 560
180	237	0.430	11 900
270	238	0.516	14 250
360	239	0.562	15 500

* based on the initial substrate concentration.

By treatment of glucoconringiin with hydrochloric acid hydroxylamine was formed which was detected by the method of Blom [5]. These results indicate that glucoconringiin is of the same structural type as the other known mustard oil glucosides [6], and has the following structure (p. 1719).

85 g of finely ground seeds of *Conringia orientalis* (L.) Andrz. were defatted by petro-



leum ether and extracted with two 1 l portions of 70 % methanol. The combined extracts were concentrated *in vacuo* to 300 ml and treated with a solution of lead acetate in order to precipitate undesired plant material. After separation of Pb-ions by H_2S the straw yellow filtrate was shortly boiled *in vacuo* until all H_2S had disappeared. The solution was filtered through a short column with 5 g of Al_2O_3 Merck and was then passed slowly through a column containing 10 ml Dowex 2-X 4 (chloride-form, 200 mesh). The effluent was free of glucoside and was discarded. After washing the resin with 200 ml of water the glucoside was eluted by 0.1 N K_2SO_4 solution. Fractions of 20 ml each were collected. The fractions 3—18 containing glucoconringiin as determined by the anthrone method and myrosinase test were brought to dryness *in vacuo*. The white residue was triturated with 3 portions of 30 ml CH_3OH at 40—50°C. The filtered methanolic solution left after evaporation *in vacuo* a colourless syrup which was dissolved in a small volume of hot 90 % ethanol. After cooling the glucoside separated partly as a viscous oil which crystallized after standing several weeks in the refrigerator. Two recrystallizations from 90 % ethanol yielded 585 mg glucoconringiin in short, white needles. F 168°C (decomp., uncorr.) $[\alpha]_D^{21}$ $-10.87°$ ($c = 3.68$; in water). UV-maximum in H_2O 230.5 mμ ($\varepsilon = 6\,720$), minimum 208 mμ ($\varepsilon = 3\,750$). (Found: C 30.81; H 4.55; N 3.52; S 14.93. Calc. for $C_{11}H_{20}NO_{10}S_2K$ (429.51): C 30.75; H 4.69; N 3.27; S 14.93.)

The identification of the products formed by enzymatic cleavage was performed in the same way as described by Kjær *et al.*[1] for the crude glucoconringiin obtained from tetraacetylglucoconringiin. From 300 ml of glucoconringiin 82 mg of crude 5,5-dimethyl-2-oxazolidinethione were obtained which gave after two recrystallizations from benzene 32 mg of pure 5,5-dimethyl-2-oxazolidinethione, melting at 107°C alone or in mixture with a synthetic sample. Glucose was determined by paper chromatography in three solvent systems; sulfate was determined as $BaSO_4$.

For the detection of hydroxylamine as a product of hydrolysis with strong acids 10 mg of glucoconringiin were dissolved in 1 ml conc. hydrochloric acid and the solution was brought to dryness on the waterbath. The residue was taken up in 2 ml of water. After addition of about 200 mg of Na acetate the solution was further treated according to the procedure described by Blom [5].

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